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A Stilbene Optical Brightener can Enhance Bacterial Pathogenicity to Gypsy Moth (Lepidoptera: Lymantriidae) and Colorado Potato Beetle (Coleoptera: Chrysomelidae)

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Stilbene optical brighteners were first investigated to protect biological control agents such as viruses, fungi, and nematodes against ultraviolet light. Some are known to enhance the activity of insect viruses in Lepidoptera. In this work, one stilbene brightener, Tinopal LPW, also increased mortality of gypsy moth and Colorado potato beetle larvae when treated with bacteria/optical brightener combinations. This increase in mortality, however, did not occur for every bacteria/insect combination. In gypsy moth, a significant increase in larval mortality was observed only with Bacillus thuringiensis *combined with Tinopal LPW. In Colorado potato beetle, however, the addition of Tinopal LPW increased larval mortality with all bacteria tested (*B. thuringiensis, Serratia marcescens, Photorhabdus luminescens*, and* Chromobacterium *sp.). The brightener also decreased the time to kill for these pathogens. This decrease in* LT_{50} *was observed not only for bacteria + Tinopal LPW combinations, but also for combinations of* Chromobacterium *sp. toxin + Tinopal LPW. The mechanism for increase in bacterial toxicity by optical brighteners is compatible with mechanisms proposed for enhancement based on viral/lepidopteran/optical brightener systems that are not dependent on replication.*

Keywords: *insect biocontrol,* Lymantria dispar, Leptinotarsa decemlineata, Bacillus thuringiensis, Serratia marcescens, Chromobacterium violaceum, Photorhabdus luminescens, *optical brightener*

INTRODUCTION

Strains of *Bacillus thuringiensis* Berliner can control a variety of insects including gypsy moth (*Lymantria dispar* (L.)) and Colorado potato beetle (*Leptinotarsa decemlineata* (Say)) in the field (Schnepf *et al*., 1998). Other bacteria such as *Serratia marcescens* Bizio (Grimont &

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Grimont, 1978) can cause low levels of mortality in insects in the laboratory, but have not been shown to control pests in the field. Strains of *Chromobacterium* sp. and *Photorhabdus luminescens* Thomas and Poinar have been discovered to be pathogenic to Colorado potato beetle (Martin, 2002).

Optical brighteners are widely used in detergents, paper, and plastics (Lanter, 1966), and as fluorescent stains for microbes (Darken, 1962). They absorb UV light and fluoresce blue, thus they were first investigated to protect biological control agents such as viruses, fungi, and nematodes against ultraviolet light (Shapiro, 1992; Nickle & Shapiro, 1994). Of numerous optical brighteners tested, several stilbenes protected LdNPV (Lymantria dispar nuclear polyhedrosis virus) 100% from UV at a concentration of 1%, and 97–100% at a concentration of 0.1% (Shapiro, 1992). An unexpected effect of feeding gypsy moth larvae a combination of an optical brightener (Tinopal LPW) and virus was increased activity of the virus (Shapiro & Robinson, 1992). The combination of virus and optical brightener reduced both the LC_{50}s as well as the LT_{50}s (Shapiro & Robinson, 1992).

Stilbene optical brighteners enhance the activity of viruses in a number of Lepidoptera (Dougherty *et al*., 1996; Shapiro, 2000). Would stilbene optical brighteners similarly enhance the activity of bacteria? To answer this question, bacterial strains that showed some toxicity to gypsy moth larvae and/or Colorado beetle larvae were selected and tested at doses near the LC_{50} whenever possible. A single optical brightener, Tinopal LPW, was chosen to test at molar concentrations near the 1 and 0.1% concentrations used for viral enhancement in Lepidoptera.

MATERIALS AND METHODS

Bacterial Strains and Media

Travers *et al*. (1987) originally isolated *B. thuringiensis* SWB from a Maryland sawdust pile by acetate selection and determined this strain to have high activity against Lepidoptera such as the cabbage looper, *Trichoplusia ni* Hübner (Travers & Martin, 1990). From a sample of Novodor (Abbott Laboratories, North Chicago, IL), we obtained *B. thuringiensis* NTEN, toxic to Colorado potato beetle. Both *B. thuringiensis* strains were grown on T3 agar (Travers *et al*., 1987) for 48 h when spores and crystals were formed (monitored by phase contrast microscopy).

M. Blackburn (USDA/ARS, Insect Biocontrol Laboratory, Beltsville, MD) provided *P. luminescens* HM, the type strain for this species. L. Carta (USDA/ARS, Nematology Laboratory, Beltsville, MD) provided *S. marcescens* TERM, originally isolated from a diseased termite colony. S. Stone (USDA/ARS, Insect Biocontrol Laboratory, Beltsville, MD) isolated *Chromobacterium* sp. PRAA from Maryland forest soil. The Gram-negative bacterial strains (*P. luminescens* HM, *Chromobacterium* sp. PRAA and *S. marcescens* TERM) were grown on L-agar (Atlas, 1997) at 25°C for 48 h.

Insects

Gypsy moths were received as egg masses from USDA/ARS, Otis Air National Guard Base (MA). Eggs were hatched and larvae reared to second instar on a wheat germ-based diet (Bell *et al*., 1981) at 25°C on a 16:8 h L:D cycle without humidity control.

The Colorado potato beetle colony originated from eggs sent from the New Jersey Department of Agriculture in 1996. The colony has been maintained on potato foliage, and field collected insects are introduced yearly to maintain genetic diversity. Colorado potato beetles were reared from eggs to second instar for bioassays on IBL (USDA/ARS, Insect Biocontrol Laboratory) potato leaf diet, a modification of the Forester diet (Gelman *et al*., 2001) made with defined ingredients as well as potato leaf powder and tomato fruit powder (Gelman *et al*., 2001). These larvae were reared in paper bags at 24°C and 46% RH.

Optical Brightener

Tinopal LPW (Calcofluor M2R, fluorescent brightener 28) was obtained from Sigma (St. Louis, MO). Tinopal LPW was dissolved in deionized water, filter-sterilized and tested at final concentrations of 10 mM (approximately 1%) or 1 mM.

***In Vitro* Testing**

Sterile 6-mm paper disks (Difco, Detroit, MI) were soaked in each concentration of Tinopal LPW for 30 min and applied to freshly spread lawns of the various bacteria along with disks soaked in water as a negative control or 30 μg/mL neomycin as a positive control. Plates were incubated for 48 h at 25°C. The diameters of the clear zones surrounding the disks were measured (to the nearest 0.1 mm) using digital vernier calipers. Each zone reported was the mean of three measurements of zones of inhibition on three separate plates.

Insect Bioassays

For gypsy moth bioassays, diet was made according to Bell *et al.* (1981). For Colorado potato beetle bioassays, the diet was made as described (Gelman *et al*., 2001) without neomycin. Both diets were poured into 96-well polypropylene molds (Bellco Glass, Inc. Vineland, NJ) and frozen overnight (-20°C). The diets were then dried in a Virtis Advantage Freeze Drier (The Virtis Co., Inc., Gardiner, NY). Dry diet pellets were sealed and stored in plastic bags at 4°C until use.

Thirty-two pellets for each treatment were placed individually in wells (1.6 cm wide × 1.6 cm deep) of white plastic bioassay trays (C-D International, Ocean City, NJ) and re-hydrated with 0.3 mL of deionized water (control), Tinopal LPW (fluorescent brightener), deionized water containing dilutions of the individual pathogen (pathogen), or the pathogen with Tinopal LPW (pathogen + fluorescent brightener). The pellets absorbed all liquid before insects were added.

We harvested each of the bacteria used in the bioassays from a single Petri dish of the appropriate medium, harvested the bacteria into 20 mL of sterile deionized water and tested each at a dilution, approximating the LC_{50}, based on previous bioassays. Since bacterial titers could only be determined after the bioassay was completed, dilutions were used as estimates. The dilutions used were: 1:10 for *B. thuringiensis* NTEN, *P. luminescens* HM, and *Chromobacterium* sp. PRAA; 1:1000 for *B. thuringiensis* SWB; and 1:2 for *S. marcescens* TERM. After re-hydration, one second instar gypsy moth or Colorado potato beetle larva was added to each pellet. Wells were sealed with film and holes made in the film with insect pins for aeration. Gypsy moth larvae were incubated in a chamber at 25°C and 16:8 h L:D cycle without humidity control. Colorado potato beetle larvae were incubated at 24°C, 16:8 h L:D cycle, 46% RH. Mortality was recorded at 24, 48, 72, 96, and 120 h after treatment. Because some of the pathogens did not cause 50% mortality alone, we examined sub-lethal effects. In these cases, the weights of the surviving larvae were measured at 6 days and mean weights were calculated by treatment. Each pathogen/optical brightener combination was replicated at least twice.

Statistics

Weights were compared by overall means. We used the MEAN and MIXED procedures (SAS, 1999) for mean comparisons with variance grouping when needed. Means were separated using a macro with $\alpha = 0.05$ using LSD (Saxton, 1998). The SAS PROBIT procedure was used to calculate LT_{50} values with 95% confidence limits (CL). A chi-square test was used to determine if the combined mortality in the bioassays in which the bacteria were mixed (observed mortality) was the same as the sum of the individual mortality of the bacteria tested separately (expected mortality).

RESULTS

In Vitro Inhibition

Neither concentration of Tinopal LPW nor the paper disks alone inhibited the growth of any of the bacteria tested. The zones of inhibition around the disks with neomycin (30 μg) measured 11.55 ± 0.14 mm for *P. luminescens* HM, 17.07 ± 0.87 mm for *S. marcescens* TERM, 17.4 ± 0.57 mm for *B. thuringiensis* NTEN, 17.51 ± 0.74 for *B. thuringiensis* SWB and 17.67 ± 0.87 mm for *Chromobacterium* sp. PRAA.

Preliminary Toxicity

For enhancement of virus activity in Lepidoptera, optical brighteners are usually used at a concentration of 1%. For Tinopal LPW, 1% is 9.6 mM, so 10 mM was used as an approximation of this value. When this concentration of Tinopal LPW was used against second instar gypsy moth larvae, 10% of the larvae died at 1 week, and the weights of the surviving larvae were reduced 44% compared to the control (control: 79.3 ± 4.6 mg; Tinopal LPW: 52.7 ± 2.8 mg). The Tinopal LPW-treated larvae fed normally and produced a similar amount of frass as the controls. On the other hand, the pathogen-treated larvae did not produce frass. When 1 mM Tinopal LPW was added to the diet, no mortality occurred and the larvae treated with Tinopal LPW were comparable in weight to the controls (control: 63.8 ± 2.1 mg; Tinopal LPW: 61.8 ± 2.4 mg). Control and Tinopal LPW mortality at 1 mM did not exceed 3% in any experiment and was usually 0.

When tested against second instar Colorado potato beetle larvae, 10 mM Tinopal LPW alone caused 79% mortality in the larvae after 96 h. A concentration of 1 mM Tinopal LPW, such as was used for gypsy moth larvae, caused no mortality above the water control (3%) when used alone against Colorado potato beetle second instar larvae. This concentration was used in further experiments.

Toxicity to Gypsy Moth Larvae

Gypsy moth larvae treated with *B. thuringiensis* SWB, at a 1:100 dilution, all died within 96 h, whereas only 15.6% of those treated with a 1:1000 dilution died. The mortality of larvae treated with *B. thuringiensis* SWB + Tinopal LPW (1 mM) at the same dose increased to 34.4% at 96 h ($\chi^2 = 28.2$, df = 3, $P < 0.01$, titer = 8.0×10^5 cell/diet pellet). The weights of the larvae treated with Tinopal LPW (1 mM) were not significantly different than the controls (Table 1), but weights of the survivors treated with *B. thuringiensis* SWB alone or *B. thuringiensis* SWB + Tinopal LPW were significantly lower than the weights of the controls. In replicate experiments, the mortality caused by the combination of *B. thuringiensis* SWB + Tinopal LPW was approximately double that of mortality caused by *B. thuringiensis* alone (40.6–75% titer = 1.1×10^5 cells/diet pellet; 31.3–51.6%, titer = 1.4×10^5 cells/diet pellet).

TABLE 1. Effects of pathogens and optical brightener on weights of gypsy moth larvae

	Weights ± standard error (mg)			
Pathogen	Control	Optical brightener	Pathogen alone	Pathogen + optical brightener
B. thuringiensis SWB	98.7 ± 3.7 A*	96.3 ± 4.6 A	34.6 ± 1.6 C	42.1 ± 2.4 B
S. marcescens TERM	160.1 ± 5.5 A	144.3 ± 6.6 A	70.2 ± 3.2 B	65.8 ± 2.8 B
Chromobacterium sp. PRAA	104.7 ± 6.6 A	120.0 ± 6.6 A	60.8 ± 3.4 B	59.3 ± 3.3 B
P. luminescens HM	65.0 ± 1.9 A	64.8 ± 1.9 A	51.9 ± 2.7 B	55.7 ± 2.2 B

Results of a typical experiment.
* Weights in the same row followed by the same letter are not significantly different (LSD, $\alpha = 0.05$).

The weights of the larvae treated with *B. thuringiensis* SWB with or without the optical brightener were also significantly less than the controls.

For *S. marcescens* TERM-treated larvae, there was also an increase in mortality with the addition of Tinopal LPW (1 mM) from 28 to 38% at 120 h ($\chi^2 = 2.125$, df = 4, $P = 0.715$, titer = 6.0×10^8 cells/diet pellet), but this increase was not significant. In replicate experiments, mortality also increased slightly with the addition of optical brightener, but not significantly (9.4–15.6% titer = 5.4×10^8 cells/pellet) The reduction in weights of the *S. marcescens* TERM-treated larvae was similar to the reduction observed in *B. thuringiensis* SWB-treated larvae. The addition of Tinopal LPW did not further reduce larval weights (Table 1).

All gypsy moth larvae fed either *Chromobacterium* sp. PRAA or *P. luminescens* HM with or without Tinopal LPW survived 120 h until weighing. Larval weights were reduced 42% (31% in replicated experiment) when larvae were fed *Chromobacterium* sp. PRAA and no further reduction in weight occurred with the addition of Tinopal LPW (Table 1). For larvae fed *P. luminescens* HM, larval weights were reduced 20%, titer = 2.1×10^7 cells/diet pellet (23% in replicated experiment, titer = 8.1×10^7) and no further reduction in weight occurred with the addition of Tinopal LPW (Table 1).

Toxicity to Colorado Potato Beetle Larvae

The mortality of Colorado potato beetle larvae treated with *B. thuringiensis* NTEN increased only from 56.6 to 59.4% at 120 h ($\chi^2 = 8.5$, df = 4, $P = 0.078$, titer = 1.2×10^6 cells/pellet; Table 2) with the addition of Tinopal LPW (1 mM). The larvae treated with *B. thuringiensis* NTEN + Tinopal LPW that died, died 24 h earlier than those treated with *B. thuringiensis* NTEN alone (Table 3). When the surviving larvae were compared at 5 days, the larvae that had fed on diet containing 1 mM Tinopal LPW were not significantly heavier (6.1 ± 0.7 mg) than the controls (4.9 ± 0.8 mg). The weights of the survivors of *B. thuringiensis* NTEN or *B. thuringiensis* NTEN + Tinopal LPW treatments were less than half the weights of the control larvae at 2.2 ± 0.1 and 2.4 ± 0.2 mg, respectively. In other

TABLE 2. Mortality of Colorado potato beetle larvae with and without optical brightener

		% Mortality		
Pathogen	Time (h)	Optical brightener	Pathogen alone	Pathogen + optical brightener
B. thuringiensis NTEN	24	0	0	0
	48	0	6.5	0
	72	0	6.5	9.4
	96	3.2	16.7	37.6
	120	6.5	56.6	59.4
S. marcescens TERM	24	0	0	9.4
	48	0	3.1	28.1
	72	3.1	6.3	50.0
	96	3.1	6.3	65.6
Chromobacterium sp. PRAA	24	0	0	0
	48	3.1	0	21.2
	72	3.1	25.7	54.5
	96	6.3	42.9	72.7
	120	6.3	62.8	87.9
P. luminescens HM	24	0	0	0
	48	0	3.1	18.8
	72	3.1	12.5	53.1
	96	9.4	40.6	71.9

TABLE 3. Effect of optical brightener on the speed of kill of Colorado potato beetle larvae by bacterial pathogens

Pathogen	LT_{50} (95% CL)*	
	Pathogen alone	Pathogen + optical brightener
B. thuringiensis NTEN	123.1 (111.0–145.9)	109.0 (102.1–121.2)
S. marcescens TERM	297.6 **	94.7 (81.7–114.6)
Chromobacterium sp. PRAA	104.2 (96.2–115.0)	76.1 (68.6–83.7)
P. luminescens HM	102.7 (92.3–126.5)	75.2 (68.0–83.7)

Results of a typical experiment.
* LT_{50} expressed in hours.
** Confidence limits could not be calculated as the LT_{50} was estimated.

experiments using less *B. thuringiensis* NTEN, the increase in mortality was slight with the addition of Tinopal LPW (6–9%; 12.5–18.8%).

Not more than 25% of Colorado potato beetle second instar larvae died when treated with a 1:2 dilution of *S. marcescens* alone. When treated with a undiluted *S. marcescens* TERM, the highest mortality for second instar larvae was 37.5%. The mortality of *S. marcescens* TERM + Tinopal LPW-treated Colorado potato beetle larvae increased from 6.5 to 65.6% ($\chi^2 = 157.3$, df = 3, $P < 0.01$, titer = 2.2×10^8 cells/pellet; Table 2). The larvae, treated with *S. marcescens* TERM + Tinopal LPW, started dying at 24 h (Table 3). In replicated experiments, similar results were obtained. In one experiment, the mortality increased from 15 to 72%, and in another, from 21.9 to 68.8%. When a 16-h culture instead of a 48-h culture was tested the mortality increased from 3.4 to 13.3%.

For *Chromobacterium* sp. PRAA-treated Colorado potato beetle larvae, the mortality increased from 62.8 to 87.9% ($\chi^2 = 63.04$, df = 4, $P < 0.01$, titer = 2.4×10^8cells/pellet; Table 2) with the addition of Tinopal LPW. Mortality of 80–90% was also obtained with a 1:2 dilution of *Chromobacterium* sp. PRAA. The larvae exposed to the *Chromobacterium* sp. PRAA + Tinopal LPW combination began dying 24 h earlier (Table 3). When weighed at 6 days, the control larvae were heavier (13.1 ± 1.1 mg) than the optical brightener treated larvae (10.6 ± 1.1 mg). The weights of the surviving larvae treated with *Chromobacterium* sp. PRAA (3.1 ± 0.5 mg) or *Chromobacterium* sp. PRAA + Tinopal LPW (4.4 ± 0.6 mg) were less than half the control weights. In replicated experiments, the increase in mortality was similar (65.6–81.55%, titer = 5.3×10^8 cells/pellet) but the larvae always died earlier.

Because no *Chromobacterium* sp. PRAA cells are recoverable from cultures greater than 10 days old, (titer < 30 cells/diet pellet), these suspensions must kill by toxin. The toxin suspensions were also tested in combination with Tinopal LPW at 1 mM. At 72 h after being exposed to a 1:10 dilution of a 10 day *Chromobacterium* sp. PRAA culture scraped from a plate, the larval mortality increased from 56 to 94% ($\chi^2 = 11.13$, df = 4, $P = 0.033$). The LT_{50} for the *Chromobacterium* sp. PRAA 10 days culture alone was 75.6 h (95% CL: 68.9–82.4), whereas the LT_{50} for the *Chromobacterium* sp. PRAA 10 days culture + Tinopal LPW combination was 48.6 h (95% CL: 43.4–53.8). In this case, only a toxin was involved as no viable cells were recovered.

We also tested the toxin of *Chromobacterium* sp. PRAA with a lower concentration of Tinopal LPW (0.5 mM) and at this concentration larval mortality increased, but not significantly, from 68.8 to 75% ($\chi^2 = 2.11$, df = 4, $P = 0.674$). However, the LT_{50} (while longer than the previous experiment for *Chromobacterium* sp. PRAA alone (85.0 h, 95% CL: 78.0–92.4)) still decreased by almost 10 h (75.6 h, 95% CL 67.7–83.5) for the *Chromobacterium* sp. PRAA/Tinopal LPW combination.

For *P. luminescens* HM-treated Colorado potato beetles, the larval mortality increased from 40.6 to 72% ($\chi^2 = 40.6$, df = 3, $P < 0.01$, titer = 6.0×10^7 cells/pellet; Table 2) with the addition of Tinopal LPW. A dilution of 1:4 of *P. luminescens* HM produced 70–80%

mortality when fed alone. The larvae also died more quickly from the *P. luminescens* HM Tinopal LPW combined treatment (Table 3). In a repeat of this experiment, the larval mortality increased from 40.6 to 53.1% with mortality occurring about 24 h earlier.

DISCUSSION

Other than *B. thuringiensis*, there have been few bacterial successes in insect control, especially for Colorado potato beetle and gypsy moth. There are many reasons for the lack of control, ranging from lack of persistence to efficacy. To determine if optical brighteners could be used to enhance mortality caused by bacteria, it was first necessary to determine if the bacteria and the specific brightener were compatible, that the brightener did not inhibit the growth of the bacteria (Martin *et al*., 1998). Tinopal LPW did not inhibit the growth of any of the pathogens tested at either the 10 or 1 mM level. Tinopal LPW concentrations of 10 mM, typically used for viruses, while not interfering with bacterial growth, caused insect mortality above control levels. This would be expected if the optical brightener induced some virus already present in the gypsy moth larvae as all the mortality was only noted late (7 days) when the insect was weighed. Optical brightener inducing indigenous virus in field trials for gypsy moth has been observed (Webb, pers. comm.). For the Colorado potato beetle, optical brightener-treated larvae a 1% concentration caused mortality beginning at 48 h, too rapid for a virus kill.

This increase in mortality of bacterial pathogens at 1 mM optical brightener was especially effective for Colorado potato beetle/pathogen/Tinopal LPW combinations. This lower concentration of optical brightener was still effective in increasing gypsy moth mortality induced by virus (Shapiro, 1995). The 10-fold increase in mortality in beetles was less than the 1000-fold enhancement seen with the gypsy moth nuclear polyhedrosis virus optical/brightener combination (Argauer & Shapiro, 1997).

In terms of toxicity, the effects of Tinopal LPW/gypsy moth/pathogen combinations compared to Tinopal LPW/Colorado potato beetle/pathogens combinations were distinct. The addition of Tinopal LPW (1 mM) significantly increased the larval mortality in gypsy moths treated with *B. thuringiensis* SWB+Tinopal LPW, but did not significantly increase the final mortality of Colorado potato beetle larvae treated with *B. thuringiensis* NTEN+Tinopal LPW compared to larvae treated with *B. thuringiensis* NTEN alone. In gypsy moth, neither *P. luminescens* HM nor *Chromobacterium* sp. PRAA caused any mortality with or without Tinopal LPW. The lack of weight gain was the only measure of the effect of these bacteria on gypsy moth larvae. On the other hand, mortality of Colorado potato beetle larvae treated with the same two pathogens (*P. luminescens* HM and *Chromobacterium* sp. PRAA)+Tinopal LPW was significantly increased over the mortality of larvae treated with these pathogens alone. *Photorhabdus luminescens* HM and *Chromobacterium* sp. PRAA as well as both *B. thuringiensis* strains kill primarily by toxins.

Enhancement of *S. marcescens* was similar in both insects. In both gypsy moth and Colorado potato beetle larvae, the *S. marcescens* TERM+Tinopal LPW treatment increased larval mortality compared to the *S. marcescens* treatment alone. In summary, for the pathogens tested, this variability of response to optical brightener makes predictions of enhancement of toxicity of novel pathogens impractical without specific testing.

Different fluorescent brighteners have been used with varying success to enhance the activity of viruses against lepidopteran larvae (Shapiro, 1995) and some combinations are more effective than others (Argauer & Shapiro, 1997). Additional experimentation with *B. thuringiensis* SWB and Blankophor P167 with gypsy moth larvae did show enhanced mortality, whereas *B. thuringiensis* and Blankophor HRS did not (data not shown). Part of the viral enhancement may be attributed to replication of the virus in cells in which it does not normally replicate (Adams *et al*., 1994; Washburn *et al*., 1998). Therefore, initially it was hypothesized that increased replication of bacteria might increase mortality. However, that the mortality caused by the *Chromobacterium* sp. PRAA toxin without viable cells was also

increased by the addition of Tinopal LPW argues against the necessity for bacterial replication.

The mode of action of optical brighteners as enhancers is not well understood. The baculovirus/gypsy moth/optical brightener combination lowers the pH of the midgut (Shepard *et al*., 1994) changing the digestion of food. Other studies on optical brighteners and insect viruses suggest that enhancement may occur by inhibition of peritrophic membrane synthesis (Wang & Granados, 2000) making the midgut more accessible to infectious agents. Inhibition of sloughing of midgut cells of Tinopal LPW/virus-treated larvae allowed the virus to replicate in those cells (Washburn *et al*., 1998). If viral replication accounts for some of the enhancement of optical brightener, then lack of increase in mortality by the some of the bacteria + brightener combination might be expected as most of the bacteria used in this study kill by toxins (Bowen *et al*., 1998; Schnepf *et al*., 1998; Martin, 2002). The increase in mortality of Tinopal LPW/pathogen combinations observed for both gypsy moth and Colorado potato beetle larvae appears to be compatible with the above-proposed modes of action for virus in Lepidoptera that do not depend on viral replication.

The Colorado potato beetle/*Chromobacterium* sp./Tinopal LPW combination may be a good model to study toxin + optical brightener enhancement because the enhanced toxicity is not confounded by bacterial replication. While, *B. thuringiensis* also kills by toxins, the spore germinates and replicates in the cadaver (data not shown), whereas *Chromobacterium* sp. PRAA does not (data not shown).

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